

CLAIMS

1. A method for strand specific amplification, comprising:
determining nucleic acid sequences of a target nucleic acid strand;
designing a convertible oligonucleotide based, at least in part, on said target nucleic acid strand;
conducting a transcription reaction utilizing said convertible oligonucleotide and said target nucleic acid strand to provide at least one resultant complementary strand;
conducting an amplification reaction to amplify said at least one resultant complementary strand; and
analyzing said amplification reaction.
2. The method of claim 1, wherein said designing step further comprises a step of conducting thermodynamic analysis of said convertible oligonucleotide to predict secondary structure of said convertible oligonucleotide under reaction conditions of at least one of said transcription reaction and under said amplification reaction.
3. The method of claim 2, wherein said predicted secondary structure of said convertible oligonucleotide provides for at least a portion thereof in a stem-loop conformation under conditions of said transcription reaction.
4. The method of claim 1, wherein said designing step further comprises the step of considering predicted secondary structures of at least a first portion and a second portion of said convertible oligonucleotide, under differing reaction conditions.
5. The method of claim 1, wherein said designing step includes selecting nucleotides to provide said convertible oligonucleotide with at least a step-loop portion and a portion for annealing to at least a portion of said target nucleic acid strand.
6. The method of claim 1, further comprising the step of providing at least one hemi-nested primer for use in said amplification reaction.

7. The method of claim 6, wherein said at least one hemi-nested primer has a T_a that is substantially similar a T_m of said a convertible oligonucleotide, under reaction conditions of said amplification reaction.

8. The method of claim 6, wherein said hemi-nested primer includes a 3' portion having added nucleotides complementary to a transcription reaction product that is itself at least in part complementary to said target sequence.

9. The method of claim 1, wherein said designing step includes designing said a convertible oligonucleotide which comprises nucleotides that are complementary to said target nucleic acid strand and non-complementary portions to said target nucleic acid strand, wherein said non-complementary portions form a first conformation structure under said transcription reaction conditions and wherein said same non-complementary portions form a second conformation structure under said amplification reactions.

10. The method of claim 9, wherein said first conformation structure has at least a stem-loop portion.

11. A convertible oligonucleotide, comprising:
a first self-annealing portion; and
a second portion complementary, at least in part, to a target nucleic acid sequence.

12. The convertible oligonucleotide of claim 11, wherein said first self annealing portion is in a stem-loop conformation under conditions of a first reaction and converts to a second substantially linear conformation under differing reaction conditions than said first reaction conditions.

13. The convertible oligonucleotide of claim 11, wherein said second portion is from about 5 about 15 nucleotides or from about 8 to about 12 nucleotides.

14. The convertible oligonucleotide of claim 12, wherein said convertible oligonucleotide content of guanine, cytosine or combination of both is equal to or greater than 50% of the total nucleotide composition.
15. The convertible oligonucleotide of claim 12, wherein said first self-annealing portion has a ΔG of about ≤ -0.5 kcal/mol under transcription reaction conditions.
16. The convertible oligonucleotide 12, wherein said differing reaction conditions are at least transcription and amplification reaction conditions.
17. A method for designing multi-conformational chimeric nucleotides and hemi-nested primers, comprising:
- designing a stem-loop chimeric reverse transcription oligonucleotide having two segments, a 5' stem-loop segment and a 3' target annealing segment
 - conducting a reverse transcription reaction utilizing said stem-loop chimeric reverse transcription oligonucleotide and a target nucleic acid sequence, wherein said stem-loop chimeric reverse transcription oligonucleotide's 3' target annealing segment anneals to said target nucleic acid sequence and said 5' stem-loop segment forms a self annealing stem-loop structure during said reverse transcription reaction;
 - subjecting reaction products of said reverse transcription reaction to an amplification reaction wherein nucleic acid sequences of said stem-loop chimeric reverse transcription oligonucleotide adopt a secondary structure sufficient to allow for hybridization of a hemi-nested primer for use in said amplification reaction; and
 - analyzing results obtained from said amplification reaction.
18. The method of claim 17, further comprising the step of calculating said stem-loop segment's free energy and T_m .
19. The method of claim 17, wherein said 3' segment's melting temperature is about ± 7 degrees centigrade of said reverse transcription reaction's temperature.

20. The method of claim 17, wherein said 3' target annealing segment has a ΔG greater than or equal to about -0.5 kcal/mole under said reverse transcription reaction conditions.

21. The method of claim 17, wherein said 5' stem-loop segment has a ΔG less than or equal to about -0.5 kcal/mole under said reverse transcription reaction conditions.

22. A stem-loop chimeric oligonucleotide, comprising;
a first portion capable of forming a self-annealing stem loop under a first set reaction conditions;
a second portion that maintains a substantially linear conformation under said same first set of reaction conditions and is capable of annealing to a target nucleic acid sequence on a particular strand of nucleic acid.

23. The stem-loop chimeric oligonucleotide of claim 22, wherein said first portion capable of forming a self-annealing stem loop and said second portion have appropriate nucleotide sequences to form a substantially linear confirmation at a second set of reaction conditions dissimilar to said first set of reaction conditions.

24. A stem-loop chimeric oligonucleotide of claim 22 or 23, wherein said first set of reaction conditions are transcription reaction conditions and said second set of reaction conditions are amplification reaction conditions.